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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 2/00	A2	(11) International Publication Number: WO 98/25650
		(43) International Publication Date: 18 June 1998 (18.06.98)

(21) International Application Number: PCT/US97/21579

(22) International Filing Date: 24 November 1997 (24.11.97)

(30) Priority Data:
60/032,839 13 December 1996 (13.12.96) US

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(81) Designated States: AU, CA, CN, JP, KR, MX, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MULTI-PURPOSE COMPOSITIONS AND METHODS OF USE IN CONTACT LENS CLEANING AND DISINFECTING SYSTEMS

(57) Abstract

Two-compartment bottle assemblies useful in preparing multi-purpose compositions containing an ophthalmically acceptable enzyme and disinfectant, methods of preparing these compositions and methods involving the use of these compositions are disclosed for cleaning and disinfecting of contact lenses.

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MULTI-PURPOSE COMPOSITIONS AND METHODS OF USE IN CONTACT LENS CLEANING AND DISINFECTING SYSTEMS

Background of the Invention

The present invention relates to the field of contact lens cleaning and disinfecting. In particular, this invention is directed to the provision of multi-purpose compositions and methods for the preparation of these compositions. The invention is also directed to methods of simultaneously cleaning and disinfecting contact lenses by using the enzyme and disinfectant containing multi-purpose compositions of the present invention.

Various compositions and methods for cleaning contact lenses have been described in the patent and scientific literature. Some of these methods have employed compositions containing surfactants or enzymes to facilitate the cleaning of lenses. The first discussion of the use of proteolytic enzymes to clean contact lenses was in an article by Lo, et al. in the Journal of The American Optometric Association, volume 40, pages 1106-1109 (1969). Methods of removing protein deposits from contact lenses by means of proteolytic enzymes have been described in many publications since the initial article by Lo, et al., including U.S. Patent No. 3,910,296 (Karageozian, et al.).

Numerous compositions and methods for disinfecting contact lenses have also been described. Those methods may be generally characterized as involving the use of heat and/or chemical agents. Representative chemical agents for this purpose include organic anti-microbials such as benzalkonium chloride and chlorhexidine, and inorganic anti-microbials such as hydrogen peroxide and peroxide-generating compounds. U.S. Patents Nos. 4,407,791

and 4,525,346 (Stark) describe the use of polymeric quaternary ammonium compounds to disinfect contact lenses and to preserve contact lens care products. U.S. Patents Nos. 4,758,595 and 4,836,986 (Ogunbiyi) describe the use of polymeric biguanides for the same purpose.

Various methods for enzymatic cleaning and disinfecting contact lenses at the same time have been proposed. Methods involving the combined use of proteolytic enzymes and peroxides to clean and disinfect contact lenses simultaneously, are described in U.S. Patent No. Re 32,672 (Huth, et al.). A representative method of simultaneously cleaning and disinfecting contact lenses involving the use of proteolytic enzymes and quaternary ammonium compounds is described in Japanese Patent Publication 57-24526 (Boghosian, et al.). The combined use of a biguanide (i.e., chlorhexidine) and liquid enzyme compositions to simultaneously clean and disinfect contact lenses is described in Canadian Patent No. 1,150,907 (Ludwig, et al.). Methods involving the combined use of dissolved proteolytic enzymes to clean and heat to disinfect are described in U.S. Patent No. 4,614,549 (Ogunbiyi). The combined use of proteolytic enzymes and polymeric biguanides or polymeric quaternary ammonium compounds is described in copending, commonly assigned United States Patent Application Serial No. 08/156,043 and in corresponding European Patent Application Publication No. 0 456 467 A2 (Rosenthal, et al.), as well as in U.S. Patent No. 5,096,607 (Mowrey-McKee, et al.).

The commercial viability of most prior enzymatic cleaning products has depended on the use of stable enzyme tablets. More specifically, the use of solid enzymatic cleaning compositions has been necessary to ensure stability of the enzymes prior to use. In order to use such compositions, a separate packet containing a tablet must be opened, the tablet must be placed in a separate vial containing a solution, and the tablet must be dissolved in order to release the enzyme into the solution. This practice is usually performed only once a week due to the cumbersome and tedious procedure and potential for irritation and toxicity.

The use of concentrated liquid enzyme compositions in combination with a diluent to clean contact lenses has been attempted in an effort to avoid the cumbersome use of enzyme tablets. Those attempts, however, have been hampered by the fact that concentrated aqueous liquid enzyme compositions are inherently unstable. When a proteolytic enzyme is placed in an aqueous solution for an extended period (i.e., several months or more), the enzyme may lose all or a substantial portion of its proteolytic activity. Steps can be taken to stabilize the compositions. For example, stabilizing agents can protect enzymes from chemical instability problems during storage in an aqueous liquid, by placing the enzymes in a dormant physical conformation. However, the use of liquid enzyme compositions, as with the use of enzyme tablet compositions described above, still requires a separate, additional mixing step each time the lens is to be simultaneously cleaned and disinfected. Furthermore, since the amount of liquid enzyme composition placed in a diluting composition is controlled by the user, user error may result in too much or too little of the concentrate being dispensed in the diluting solution.

The following patents may be referred to for further background concerning prior attempts to stabilize concentrated liquid enzyme formulations: U.S. Patents Nos. 4,462,922 (Boskamp); 4,537,706 (Severson); and 5,089,163 (Aronson). These patents describe detergent compositions containing enzymes. U.S. Patent No. 5,281,277 (Nakagawa) and Japanese Kokai Patent Applications Nos. 92-370197; 92-143718; and 92-243215 describe liquid enzyme compositions for treating contact lenses.

A number of multi-purpose compositions for cleaning, disinfecting and storing contact lenses are commercially available. The main cleaning ingredients of these products generally comprise surfactants. Soft contact lenses become soiled by collecting various debris and also by accumulated protein deposition on the lens surface. Failure to remove the protein deposits results in opacification of the lens and lens spoilage. While surfactants are used to remove

debris from the lens, they are not very efficacious in removing protein deposits. Proteolytic agents, in contrast, are very effective in removing protein deposits that form on the lens over time. Thus, cleaning regimens using multi-purpose compositions comprising surfactants still require the additional step of employing a proteolytic agent to remove protein deposits.

The use of a single enzyme containing multi-purpose solution for the cleaning and disinfecting of contact lenses has been proposed in U.S. Patent No. 5,409,546 (Nakagawa et al.) and European Patent Application No. 0 646, 641 (Nakayawa et al.). These patents disclose compositions wherein the enzyme is in a dilute concentration, and the compositions, therefore, require no dilution step prior to use. These compositions, however, provide limited stability of the enzyme (1 or 2 months at room temperature). The limited shelf-life of these compositions generally does not permit their commercialization.

Brief Description of the Drawings

FIG. 1 is a perspective view of a preferred embodiment of the invention.

FIG. 2 is a elevation view of a preferred embodiment of the invention.

FIG. 3 is an exploded elevation view of a preferred embodiment of the invention.

FIG. 4 is an exploded cross-section view of a preferred embodiment of the invention about line 4-4 of FIG. 3.

FIG. 5 is a cross-section view about line 5-5 of FIG 2 of a preferred embodiment of the invention.

FIG. 6 is a cross-section view about line 5-5 of FIG. 2 of a preferred embodiment of the invention, illustrating the downward rotation of a cap/plunger assembly, the breaking of a membrane, and the egress of an enzyme composition.

FIG. 7 is a top plan view of a housing of the invention.

FIG. 8 is a bottom plan view of a cap and collar of the invention.

Summary of the Invention

The present invention is directed to two-part systems which provide for the generation of multi-purpose compositions useful in the simultaneous cleaning and disinfecting of contact lenses. The present invention is also directed to methods of simultaneously cleaning and disinfecting contact lenses using the two-part system. The two-part system comprises an enzyme cleaning composition, an aqueous composition and one or more anti-microbial agent(s). The enzyme composition provides a concentrated amount of an enzyme. The aqueous composition provides a diluting solution. The anti-microbial agent is contained in either the enzyme composition or the aqueous composition. The two-part system is initialized for use by admixing the enzyme composition with the aqueous composition.

The two-part system uses a two-compartment device capable of keeping separate an enzyme composition and a diluting composition prior to their initial use. One feature of this device is that it combines the separate components in a single bottle assembly. This feature has the advantage over prior art systems which have required the more difficult, tedious and cumbersome use of separate containers. Related to this feature is the fact that the enzyme is added only once to the disinfecting composition, and the resulting multi-purpose composition can then be used many times over a period of several months. With most prior art systems, the enzyme must be added to the disinfecting composition each time the user cleans his lenses. A further advantage of this feature is that the two compositions are admixed aseptically. This is due to the fact that the bottle assembly containing the two compositions is assembled in an

aseptic manner with an air-tight seal, thus sterile mixing is performed within the closed sterile system of the bottle assembly. Still another advantage of this feature is that it eliminates possible user error resulting from the addition of improper amounts of an enzyme composition to the diluting solution. This is important as improper amounts of enzymes or excipients (e.g., salts) in the resultant multi-purpose solution can lead to ineffective cleaning and disinfecting of the lens and/or ocular toxicity. Thus, user compliance with the cleaning regimen is perfected with the present invention, allowing for the realization of maximum cleaning benefits, and the avoidance of unnecessary ocular irritation/toxicity.

Another feature of the present invention is that the enzyme component is kept separate from the diluting solution prior to initial use. This feature minimizes enzyme activity loss, which naturally occurs over time in aqueous environments, by minimizing the time the enzyme is solubilized prior to initial use. This feature allows for ambient temperature shipping, and long shelf-life without significant loss of enzyme activity. When the user is ready to use the system, the two components are combined and mixed aseptically, forming the multi-purpose composition. The multi-purpose composition can then be used for a period of from about 1-3 months.

The cleaning and disinfecting compositions of the present invention may utilize ingredients similar to known cleaning or disinfection formulations. Various modifications may be made, however, to enhance the anti-microbial efficacy of the multi-purpose composition. Other additional components may also be added to enhance the shelf-life of the mixed components, such as the use of enzyme stabilizers.

The multi-purpose compositions and methods of the present invention provide greater ease of use. This ease of use enables contact lens users to clean their lenses daily, thereby achieving maximum proteolytic cleaning of their lenses. It has been found that daily use of the

liquid enzyme compositions in multi-purpose compositions of the present invention results in dramatically better cleaning, as compared to the once-a-week enzyme cleaning regimens currently being used.

Detailed Description of the Invention

The present invention is directed to the use of a sterile two-part system for the preparation of multi-purpose compositions useful for cleaning and disinfecting contact lenses. The present invention is also directed to methods of cleaning and disinfecting contact lenses by using a two-part system of the present invention. The first part ("Part I") is a sterile enzyme containing solid (powder or tablet) or liquid composition and the second part ("Part II") is a sterile diluting composition. An anti-microbial agent is further required, and may be included in either composition.

The present invention requires the use of a two-compartment device to store and mix the sterile two-part system, and to dispense the resultant sterile multi-purpose composition. Various devices may be employed, but the central features of the device are that it provides separate component storage, a means for aseptically adding one component to the other component, a mixing chamber and a dispensing means, all in a single bottle assembly.

Figure 1 illustrates a preferred two-part bottle assembly for use with the two-part/multi-purpose compositions of the present invention. The preferred two-part bottle assembly, bottle assembly 1, generally comprises bottle 2 and container 4.

As illustrated in FIGS. 2-4, bottle 2 comprises neck 13, opening 10, external rings 12 and internal rings 43. Neck 13 is formed such that rings 12 annularly protrude from neck 13.

Bottle 2 is made generally of molded polyethylene, although other materials such as polyethyleneterphlalate (PET) and polypropylene (P/P) may be used.

As best seen in FIGS. 3 and 4, container 4 comprises housing 5, plunger 6, cap 8 and collar 28. Housing 5 comprises hollow cylinder 14 and cap 16. Cylinder 14 has external threads 36 annularly protruding near top end 15 and membrane disk 18 covering bottom end 17. Stops 23 are disposed annularly about exterior 38 of cylinder 14 (FIG. 7). Membrane 18 has thin cross-sectional thickness 49 about its circumference. Cap 16 has protruding internal rings 20, and cylinder 14 is coaxially disposed within cap 16 such that top end 15 and bottom end 17 project out of cap 16. Plunger 6 comprises hollow cylinder 24, open end 22, dispensing end 25 and ribs 34. Open end 22 has tooth 39 and is thinner in cross-sectional thickness than the thickness of hollow cylinder 24, thereby forming sharp point 41 of tooth 39. Cap 8 comprises pin 33, hollow cone 32, internal threads 30 and stops 21. As best seen in FIG. 8, collar 28 has tab 29, spokes 19 and perforation 27 which forms ends 31. Container 4 components are generally made of molded high density polyethylene or polycarbonate, but other materials and methods of manufacture such as P/P, PET, polystyrene and acrylonitrile butadiene styrene (ABS) may be employed.

As illustrated in FIG. 4, container 4 is put together by first adding enzyme cleaning composition 7 to hollow cylinder 14 of housing 5, ringing collar 28 over cylinder 14, inserting plunger 6 within cylinder 14 such that rings 34 of plunger 6 compress against the interior of cylinder 14, and screwing on cap 8 over plunger 6, engaging pin 33 of cap 8 with notch 26 of plunger 6, by threading internal threads 30 within threads 36 of housing 5. In this configuration, spokes 19 of collar 28 are interspersed between both stops 21 of cap 8 and stops 23 of housing 5. Cap 8 in this configuration (see FIGS. 2 and 5), is only partially threaded within threads 36 of housing 5 due to the prevention of further downward rotation of cap 8 by

collar 28. Aqueous composition 9 is added to bottle 2, container 4 is then placed over neck 13, and cap 16 is forced down on neck 13 such that rings 20 compress radially against neck 13, and exterior rings 12 compress against interior 47 of cap 16, forming an air-tight seal.

In operation, collar 28 is first removed from container 4 by screwing cap 8 downward on housing 5. When cap 8 is rotated, stops 21 engage and pull spokes 19, while stops 23 hold spokes 19 stationary. The resulting stress causes collar 28 to split at perforation 27. Split collar 28 may then be removed by pulling on tab 29. Cap 8 is then further screwed down on housing 5. With cap 8 rotation, plunger 6, is simultaneously pushed downward causing plunger 6 to descend cylinder 14. When plunger 6 reaches membrane 18 of housing 5, sharp point 41 punctures thin circumference 49 of membrane 18. Further rotation of cap 8 causes open end 22 of plunger 6 to further descend, slicing off membrane 18 about its circumference from housing 5, similar to the operation of a punch press. At this point the enzyme cleaning composition contained in housing 5 is exposed to interior 11 of bottle 2 and falls into aqueous diluting composition 9 of bottle 2. Bottle 2 may then be inverted and shook, thus affecting the mixing of the enzyme and aqueous diluting compositions. When membrane 18 is cut away from housing 5, a channel is formed which runs from bottle 2 through now open housing 5, plunger 6 and dispensing end 25. With the removal of cap 8, the resultant multi-purpose composition may now be dispensed through this channel to an appropriate container for cleaning, disinfecting, rinsing and storing the contact lens.

Other embodiments of two-compartment bottle assemblies may be employed in the present invention. For example, a blister pouch and piercing means may be utilized as the enzyme compartment and break-away membrane component, respectively, of a bottle assembly.

As stated above, the present invention is comprised of two separate compositions which are then combined before initial use. Part I comprises an enzyme and Part II comprises an aqueous diluting solution. The resultant multi-purpose composition may contain various other agents, but must contain: 1) an anti-microbial agent, 2) an enzyme, 3) a buffering agent, 4) a tonicity agent, and 5) water. The multi-purpose compositions of the present invention are intended to function as storing, rinsing, cleaning and disinfecting solutions. Therefore, the multi-purpose compositions will be physiologically compatible with the eye.

The Part I sterile enzyme composition of the present invention is generally composed of one or more enzymes and various carriers. The enzyme composition may be formulated as a powder, tablet or liquid. Dry powder or tablet compositions may be preferred when the Part I enzyme compositions need to be stable for longer periods of time than liquids. Excipients which make up the enzyme powder compositions are known in the art. Generally, the enzyme powder composition will include bulking agents to carry the relatively small volume of enzyme into the diluting solution. Such bulking agents typically include polyols (e.g., mannitol or soribitol), polyethylene glycols (molecular weights greater than 1000) and sugars. Other excipients may include salts such as NaCl, chelating agents such as EDTA, and buffering agents such as Tris. Other additives may include surfactants to ease dispersion and dissolution of the powder in water. Preferred enzyme powder compositions comprise mannitol and polyethylene glycol-5000 (PEG-5000).

Enzyme tablet compositions and methods of manufacturing are known in the art. Enzyme tablets require the use of bulking agents and binding agents. Additionally, tablets may contain effervescing agents such as bicarbonate to expedite dissolution of the tablet into the diluting solution. Other excipients known in the art may be added to provide greater

consistency and easier manufacture of the tablets. Preferred enzyme tablet compositions comprise sodium bicarbonate, citric acid, PEG-8000, carboxymethyl cellulose and lactose.

Liquid enzyme compositions are preferred Part I compositions of the present invention due to their ease of preparation, sterilization and dispensing within the enzyme container of a bottle assembly. Liquid enzyme compositions and methods of manufacturing are known in the art. Enzymes contained in Part I liquid compositions may be solubilized in aqueous compositions or dispersed in non-aqueous compositions.

Aqueous enzyme compositions are generally preferred due to their ease of preparation and sterilization. Aqueous enzyme compositions typically comprise one or more polyol(s) and a borate or boric acid compound. Preferred aqueous enzyme compositions of the present invention comprise a 2-3 carbon polyol and a borate or boric acid compound. As used herein, the term "2-3 carbon polyol" refers to a compound with 2 to 3 carbon atoms and at least two hydroxy groups. Examples of 2-3 carbon polyols are glycerol, 1,2-propane diol ("propylene glycol"), 1,3-propane diol and ethylene glycol. Glycerol is the most preferred 2-3 carbon polyol. The borate or boric acid compounds which may be utilized in the liquid enzyme compositions of the present invention include alkali metal salts of borate, boric acid and borax. Other excipients which may be included in the Part I aqueous enzyme compositions include divalent ions such as calcium, enzyme stabilizing organic acids such as benzoic acid and surfactants such as alkylethoxylates.

Non-aqueous enzyme compositions employed as Part I compositions of the present invention generally comprise a crystalline enzyme uniformly dispersed in a water-soluble organic liquid. Typical organic liquids include polyoxyethylenes (e.g., PEG-400) and alkoxy polyoxyethylenes such as methoxy polyethylene glycols. In this composition, the enzyme is in a dormant state, and following dissolution in a Part II composition of the present invention, the

enzyme solubilizes and becomes active. Preferred non-aqueous enzyme compositions comprise an enzyme in PEG-400.

As stated above, the anti-microbial agent(s) of the present invention may be included in any of the enzyme compositions described herein. The actual amount of anti-microbial agent will vary, but will provide effective disinfection of the contact lens, as described below.

The above-described Part I enzyme composition are aseptically processed generally by sterile filtering the liquids, or lyophilization, aseptic processing and terminal gamma irradiation of the solids. These and other methods of sterilizing liquids or solid compositions are well known in the art.

The enzymes which may be utilized in the compositions and methods of the present invention include all enzymes which: (1) are useful in removing deposits from contact lenses; (2) cause, at most, only minor ocular irritation in the event a small amount of enzyme comes in contact with eye; (3) are relatively chemically stable and effective in dilute saline solutions; and (4) do not adversely affect the physical or chemical properties of the lens being treated. The proteolytic enzymes used herein must have at least a partial capability to hydrolyze peptide-amide bonds in order to reduce the proteinaceous material found in lens deposits to smaller water-soluble subunits. Additionally, such enzymes may exhibit some lipolytic, amylolytic or related activities associated with the proteolytic activity and may be neutral, acidic or alkaline. Furthermore, separate lipases or carbohydrases may be used in combination with the proteolytic enzymes. For purposes of the present specification, enzymes which satisfy the foregoing requirements are referred to as being "ophthalmically acceptable."

Examples of ophthalmically acceptable proteolytic enzymes which may be utilized in the present invention include, but are not limited to: pancreatin, trypsin, subtilisin, collagenase,

keratinase, carboxypeptidase, bromelain, aminopeptidase, elastase, Aspergillo peptidase, pronase E (from S. griseus), dispase (from Bacillus polymyxa) and mixtures thereof.

Microbially derived enzymes, such as those derived from Bacillus, Streptomyces, and Aspergillus microorganisms, represent a preferred type of enzyme which may be utilized in the present invention. Of this sub-group of enzymes, the most preferred are the Bacillus derived neutral or slightly alkaline proteases generically called "subtilisin" enzymes.

The identification, separation and purification of enzymes is known in the art. Many identification and isolation techniques exist in the general scientific literature for the isolation of enzymes, including those enzymes having proteolytic and mixed proteolytic/lipolytic/amylolytic activity. The enzymes contemplated by this invention can be readily obtained by known techniques from plant, animal or microbial sources.

With the advent of recombinant DNA techniques, it is anticipated that new sources and types of stable proteolytic enzymes will become available. Such enzymes should be considered to fall within the scope of this invention so long as they meet the criteria set forth herein. Preferred genetically modified enzymes include BPN' subtilisin variants, such as those described in PCT/US94/10020 (Procter and Gamble), WIPO Publication WO 95/30011 (Procter and Gamble) and U.S. Patent No. 4,990,452 (Genex Corp.). Specific preferred subtilisins and variants include subtilisin Carlsberg, subtilisin PB92, subtilisin 309, subtilisin 147, subtilisin 168, subtilisin DY and truncations, modifications and variants thereof.

Other enzymes modified from their native structure may also be used in the present invention. Such modifications include "pegylation," i.e., covalent bonding of polyoxyethylene glycol derivatives to the enzymes, as well as monomeric covalent additions to the enzymes with small organic compounds (e.g., methyl, ethyl, succinyl groups, and so on). These modifications will generally prevent the enzymes from ionically binding to the contact lens,

especially in the case of negatively charged hydrophilic soft lenses. Binding to the contact lens can expose unnecessarily high amounts of the enzyme to the eye. With some enzymes, this exposure can cause undesirable inflammatory responses. These modifications may also improve the stability of enzymes in aqueous environments such as the Part I or multi-purpose compositions of the present invention. It is believed that alkylation of hydrolytically sensitive cites of the enzyme limits the enzyme's autolysis.

Subtilisin and trypsin are preferred enzymes, and genetically modified subtilisin BPN's are the most preferred enzyme for use in the present invention. Subtilisin is derived from Bacillus bacteria and is commercially available from various commercial sources including Novo Industries (Bagsvaerd, Denmark), Fluka Biochemika (Buchs, Switzerland) and Boehringer Mannheim (Indianapolis, Indiana, U.S.A.). Trypsin is purified from various animal sources and is commercially available from Sigma Chemical Co. and Boehringer Mannheim. Subtilisin BPN' variants, as described above are genetically modified subtilisins which have been described in 4,990,452 (Genex Corp.), PCT/US94/10020 (Procter and Gamble) and WIPO Publication WO 95/30011 (Procter and Gamble). These enzymes may be obtained by methods described in those publications.

Pancreatin is extracted from mammalian pancreas, and is commercially available from various sources, including Scientific Protein Laboratories (Waunakee, Wisconsin, U.S.A.), Novo Industries (Bagsvaerd, Denmark), Sigma Chemical Co. (St. Louis, Missouri, U.S.A.), and Boehringer Mannheim (Indianapolis, Indiana, U.S.A.). Pancreatin USP is a mixture of proteases, lipases and amylases, and is defined by the United States Pharmacopoeia ("USP"). The most preferred form of pancreatin is Pancreatin 9X. As utilized herein, the term "Pancreatin 9X" means a filtered (0.2 microns) pancreatin containing nine times the USP protease unit content.

The Part I enzyme concentration will depend on various factors, such as: the enzyme or combination of enzymes selected; the quantity of enzyme composition to be added to the Part II aqueous composition; the purity, specificity and efficacy of the enzyme(s) selected; the volume of the Part II aqueous composition; the type of lenses to be cleaned; and the intended duration of each cleaning.

During storage, some of the activity of the enzyme may be lost, depending on length of storage and temperature conditions. Thus, the Part I enzyme compositions of the present invention may be prepared with initial amounts of enzyme that exceed the amount necessary to achieve the final multi-purpose enzyme concentration ranges described herein. In general, the Part I enzyme compositions of the present invention will preferably contain one or more enzymes in an amount of from about 100-100,000 PAU/g or 100-100,000 PAU/mL.

The Part I enzyme compositions, however, will contain an effective amount of one or more enzyme(s) sufficient to remove substantially or reduce significantly deposits of proteins, lipids, mucopolysaccharides and other materials typically found on human-worn contact lenses when a relatively small amount of a Part I enzyme composition is mixed with a Part II aqueous diluting composition. As used herein, such a final enzyme concentration of the resultant multi-purpose composition of the present invention is referred to as "an amount effective to clean the lens." However, the cleaning methods of the present invention will generally employ an amount of the above-described enzyme compositions sufficient to provide a final enzyme concentration in the multi-purpose composition of from about 1-100 PAU/mL of solution, following dispersion of a Part I enzyme composition in a Part II composition. A final concentration of about 5-25 PAU/mL is preferred. For purposes of this specification, a "proteolytic activity unit" or "PAU" is defined as the amount of enzyme activity necessary to

generate one microgram (mcg) of tyrosine per minute ("mcg Tyr/min"), as determined by the casein-digestion, colorimetric assay described below.

Casein-digestion assay

A 5.0 mL portion of casein substrate (0.65% casein w/v) is equilibrated for 10 minutes (min) ± 5 seconds (sec) at 37°C. An enzyme solution is prepared from a Part I enzyme composition by solubilizing and diluting the Part I composition in PBS buffer. A 1.0 mL portion of this enzyme solution (0.2 mg/ml) is then added to the casein substrate and the mixture vortexed, then incubated for 10 min ± 5 sec at 37°C. After incubation, 5.0 mL of 14% trichloroacetic acid is added and the resultant mixture immediately vortexed. The mixture is incubated for at least another 30 min, then vortexed and centrifuged for 15-20 min (approx. 2000 rpm). The supernatant of the centrifuged sample is filtered into a serum filter sampler and a 2.0 mL aliquot removed. To the 2.0 mL sample is added 5.0 mL of 5.3% Na_2CO_3. The sample is vortexed, 1.0 mL of 0.67 N Folin's Phenol reagent is added, and the sample is immediately vortexed again, then incubated for 60 min at 37°C. The sample is then read on a visible light spectrophotometer at 660 nanometers (nm) versus purified water as the reference. The sample concentration is then determined by comparison to a tyrosine standard curve. The Part I concentration is then calculated by taking into account the dilution ratio.

The Part II aqueous compositions provide the volume of distilled water necessary for the multi-purpose compositions of the present invention. In general, the Part II composition may also contain sodium chloride and other excipients which together provide an ophthalmically compatible solution. However, as noted above, Part I compositions may contain a percentage or all of these ingredients, and Part II compositions may provide only a

percentage of these ingredients, or none at all. As will be appreciated by those skilled in the art, the Part II compositions utilized in the present invention may contain various other components such as suitable buffering agents, chelating and/or sequestering agents and tonicity adjusting agents. The Part II compositions may also contain surfactants. As stated above, the anti-microbial agent may also be included in the Part II compositions. In general, the Part II compositions will contain one or more anti-microbial agents (e.g., PHMB or polyquaternium-1), a buffer (e.g., borate), citrates, tonicity agents (e.g., NaCl, sugars), a chelating agent (e.g., EDTA), and surfactants (e.g., block copolymers). Other agents which enhance the anti-microbial efficacy of the compositions, such as amino alcohols and alkylamines, may also be added. Preferred Part II compositions comprise polyquaternium-1, sodium borate, boric acid, propylene glycol and Pluronic P-103The most preferred Part II compositions comprise boric acid, sorbitol, 95% 2-amino-2-methyl-1-propanol ("AMP-95"), sodium citrate, sodium chloride, disodium edetate, polyquaternium-1, Tetronic 1304 and myristamidopropyl dimethyl amine ("MAPDA").

The multi-purpose compositions are intended to be used with various types of contact lenses including rigid gas-permeable ("RGP") lenses and soft lenses. Depending on the type of lens to be cleaned, the multi-purpose compositions may be optimized to effect maximum cleaning benefits while minimizing ocular irritation/toxicity potential. For example, as soft contact lenses are electronegatively charged, they tend to bind enzymes with high isoelectric points (i.e., more positively charged at the pH of a multi-purpose composition). This greater exposure of enzyme to the eye, via an enzyme loaded lens, may lead to ocular irritation/toxicity. Thus, the use of native or modified enzymes having lower isoelectric points (e.g., subtilisin BPN' variants), and therefore less tendency for interaction with the soft lenses,

is preferable. Less consideration of enzyme choice is necessary when RGP lenses are to be treated, due to generally less enzyme binding potential of RGP lenses.

The cleaning obtained with the liquid enzyme compositions of the present invention is a function of the time. The soaking times utilized will generally vary from about 1 hour to overnight. However, if longer soaking periods (e.g., 24 hours) were to be employed, lower concentrations than those described above may be utilized.

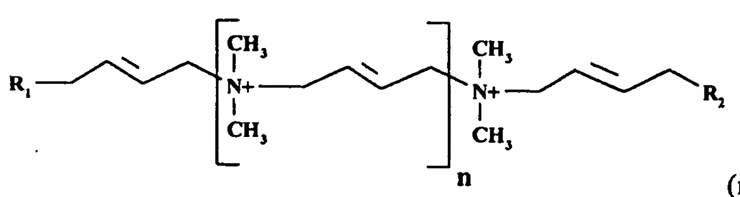
The cleaning methods of the present invention involve the use of a small amount of the above-described enzyme compositions to facilitate the removal of proteins and other deposits from contact lenses. The amount of enzyme composition used in particular embodiments of the present invention may vary, depending on the enzyme concentration, as described above, as well as various other factors, such as the purity of the enzyme, the proposed duration of exposure of lenses to the compositions, the nature of the lens care regimen (e.g., the frequency of lens disinfection and cleaning), the type of lens being treated, and the use of adjunctive cleaning agents (e.g., surfactants). In general, about 1 gram of powder, 1 tablet or 1 milliliter of a Part I compostion will be added to about 120 mL of a Part II composition, although greater or lesser amounts are contemplated by the present invention.

The enzyme compositions of the present invention will demonstrate effective cleaning efficacy while exhibiting minimal adverse effects or, more preferably, enhanced effects on the anti-microbial activity of anti-microbial agents. The anti-microbial activity of disinfecting agents, particularly polymeric quaternary ammonium compounds such as polyquaternium-1, is adversely affected by high concentrations of sodium chloride or other ionic solutes. More specifically, polymeric quaternary ammonium compounds, and particularly those of Formula (I), below, lose anti-microbial activity when the concentration of ionic solutes in the multi-purpose compositions is too high. Generally, the multi-purpose compositions of the present

invention will have tonicities/osmolalities in the range of hypotonic to isotonic, and more preferably in the range of 150 to 350 milliOsmoles per kilogram (mOs/kg). A range of 200 to 300 mOs/kg is particularly preferred, and an osmolality of about 220 mOs/kg is most preferred.

The cleaning and disinfecting methods of the present invention utilize a multi-purpose composition of the present invention containing an anti-microbial agent. Anti-microbial agents will generally be non-oxidative polymeric anti-microbial agents which derive their anti-microbial activity through a chemical or physicochemical interaction with the organisms. As used in the present specification, the term "polymeric anti-microbial agent" refers to any nitrogen-containing polymer or co-polymer which has anti-microbial activity. Preferred polymeric anti-microbial agents include: polyquaternium-1, which is a polymeric quaternary ammonium compound; and polyhexamethylene biguanide ("PHMB") or polyaminopropyl biguanide ("PAPB"), which are polymeric biguanides. These preferred anti-microbial agents are disclosed in U.S. Patent Nos. 4,407,791 and 4,525,346, issued to Stark, and 4,758,595 and 4,836,986, issued to Ogunbiyi, respectively. The entire contents of the foregoing publications are hereby incorporated in the present specification by reference. Other anti-microbial agents suitable in the methods of the present invention include: other quaternary ammonium compounds, such as benzalkonium halides, and other biguanides, such as chlorhexidine. The anti-microbial agents used herein are preferably employed in the absence of mercury-containing compounds such as thimerosal.

The most preferred anti-microbial agents are polymeric quaternary ammonium compounds of the structure:



$(n + 1)X^-$ (I)

wherein:

R_1 and R_2 can be the same or different and are selected from:
$N^+(CH_2CH_2OH)_3X^-$,
$N(CH_3)_2$ or OH;
X^- is a pharmaceutically acceptable anion, preferably chloride; and
n = integer from 1 to 50.

The most preferred compounds of this structure is polyquaternium-1, which is also known as Onamer M™ (registered trademark of Onyx Chemical Corporation) or as Polyquad® (registered trademark of Alcon Laboratories, Inc.). Polyquaternium-1 is a mixture of the above referenced compounds, wherein X^- is chloride and R_1, R_2 and n are as defined above.

The above-described anti-microbial agents are utilized in the methods of the present invention in an amount effective to eliminate substantially or to reduce significantly the number of viable microorganisms found on contact lenses, in accordance with the requirements of governmental regulatory agencies, such as the United States Food and Drug Administration. For purposes of the present specification, that amount is referred to as being "an amount effective to disinfect" or "an anti-microbially effective amount." The amount of anti-microbial agent employed will vary, depending on factors such as the type of lens care regimen in which the method is being utilized. For example, the use of an efficacious daily cleaner in the lens care regimen may substantially reduce the amount of material deposited on the lenses, including microorganisms, and thereby lessen the amount of anti-microbial agent required to disinfect the lenses. The type of lens being treated (e.g., "hard" versus "soft" lenses) may also be a factor. In general, a concentration in the range of about 0.00001% to about 0.01% by weight of one or more of the above-described anti-microbial agents will be employed. The

most preferred concentration of the polymeric quaternary ammonium compounds of Formula (I) is about 0.001% by weight.

The methods of the present invention will typically involve adding about 2-10 mL of a multi-purpose composition of the present invention to a lens case, placing the soiled lens into the dispensed multi-purpose composition, and soaking the lens for a period of time effective to clean and disinfect the lens. Optionally, the contact lenses are first rubbed with a multi-purpose composition of the present invention or a surfactant cleaner prior to immersion in the multi-purpose composition. The lens will typically be soaked overnight, but shorter or longer durations are contemplated by the methods of the present invention. A soaking time of 4 to 8 hours is preferred. The methods of the present invention allow the above-described regimen to be performed daily.

The following examples are presented to illustrate further, various aspects of the present invention, but are not intended to limit the scope of the invention in any respect.

Example 1

A preferred Part I enzyme composition and a preferred Part II aqueous composition containing an anti-microbial agent for use in a two-component bottle assembly/multi-purpose composition of the present invention, are described below:

A. Part I Liquid Trypsin Composition

The following liquid enzyme composition represents a preferred enzyme composition of the present invention:

Ingredient	Amount
Enzyme	3000 PAU/mL
Boric acid	1.0% (w/v)
PEG-400	25% (w/v)
Glycerol	25% (w/v)
Calcium chloride	0.25% (w/v)
Sodium hydroxide	QS to adjust pH to 5 to 8
Hydrochloric acid	QS to adjust pH to 5 to 8
Purified water	QS

Calcium chloride and boric acid are dispersed in 30% of the volume of purified water. PEG and glycerol are then added. The pH of the solution is adjusted, and the enzyme is then dissolved in the solution, followed by a final volume adjustment with purified water. The composition is the sterile filtered using a 0.2 μm filter.

B. Part II Aqueous Composition

The following formulation represents a preferred aqueous composition:

Ingredient	% (w/v)
Polyquaternium-1	0.001
Boric acid	0.6
Sodium chloride	0.1
AMP-95	0.45
MAPDA	0.0005
Sorbitol	1.2
Sodium citrate	0.65
Tetronic 1304	0.05
Disodium Edetate	0.05
Sodium hydroxide	To adjust pH 6.5 to 8.0
Hydrochloric acid	To adjust pH 6.5 to 8.0
Purified water	QS

The ingredients are dissolved with 90% of the volume of purified water, the pH is adjusted, and the volume is then brought up to 100% volume. The composition is then sterile filtered using a 0.2 μm membrane filter.

Various volumes of the above enzyme and aqueous compositions may be employed in a two-compartment bottle assembly of the present invention. Preferred amounts include 1 mL of the enzyme composition and 120 mL of the aqueous composition.

Example 2

The following is an example of a liquid enzyme composition of the present invention:

Ingredient	Amount
Trypsin	2200 PAU/mL
Sodium borate	7.6% (w/v)
Propylene glycol	50% (v/v)
NaOH/HCl	QS to pH 5 to 8
Water	QS

The above formulation is prepared by first sequentially mixing propylene glycol, purified water, hydrochloric acid and sodium borate together. The required amount of trypsin (about 0.3 w/v) is then dissolved the above mixture, the pH is adjusted and the solution is brought to 100% volume. The enzyme composition is then sterile filtered (0.2 μm filter). The optimal pH of the above formulation will be in the range of 5-7; a pH of 6 is most preferred.

Example 3

The following are examples of preferred Part I solid enzyme compositions of the present invention.

I. Part I Trypsin Tablet Composition

Ingredient	Amount
Trypsin	4000 PAU/mg
Sodium bicarbonate	8.5 mg
Citric acid	3.5 mg
PEG-3350	3.0 mg
Lactose	QS to 50 mg

II. Part I Subtilisin Tablet Composition

Ingredient	Amount
Subtilisin	2000 PAU/mg
Sodium bicarbonate	16 mg
Citric acid	6.5 mg
PEG-3350	6.0 mg
Mannitol	QS to 80 mg

The tablets are generally prepared by first mixing the appropriate amounts of each of the ingredients and then passing the mixture through an oscillating granulator equipped with a 20 mesh hard screen. The screened ingredients are then added to a suitably sized blender and mixed for 30 minutes. An appropriate amount of PEG and enzyme are then passed through a 20 mesh hard screen and this mixture is then added to the blender. The combined screened ingredients are then blended for an additional 15 minutes. Using a tablet press equipped with a 5/32" tooling, the blended ingredients are then compressed into tablets having a target weight of 50-80 mg and a hardness of 8 SCU. The tablets may then be sterilized by the method of gamma-sterilization.

III. Part I Trypsin Powder Composition

Ingredient	Amount
Trypsin	3000 PAU (~ 3-4 mg)
Lactose	QS to 1 g

The enzyme and lactose are dissolved in water (1g of enzyme/lactose per 1 mL of water) and sterile filtered using a 0.2 μm filter. The sterile enzyme solution as then aseptically lyophilized.

Example 4

The following are examples of Part II aqueous compositions of the present invention:

I. Part II Aqueous Composition

Ingredient	Amount (w/v)
Polyquaternium-1	0.0002%
Sodium borate	0.25%
Propylene glycol	1.0%
Pluronic P-103	0.1%
Sodium hydroxide	To adjust pH to 6.5 to 8.0
Hydrochloric acid	To adjust pH to 6.5 to 8.0
Purified water	QS

II. Part II Aqueous Composition

Ingredient	Amount (w/v)
PHMB	0.0001%
Sodium phosphate	0.28%
Potassium phosphate	0.06%
Sodium chloride	0.7%
Disodium edetate	0.05%
Sodium hydroxide	to adjust to pH 6.5 to 8.0
Hydrochloric acid	to adjust to pH 6.5 to 8.0

These Part II compositions are prepared in a similar way as those of Example 1, above.

The Part I and II compositions described in the Examples above will be combined, stored and mixed in a single bottle assembly in various quantities. In general, preferred amounts will be:

Part I: 1 g of powder or 1 tablet of a solid enzyme composition, or 1 ml of liquid enzyme composition.

Part II: about 120 ml

(Similarly 2 g of powder, 2 tablets or 2 ml of liquid would be combined with about 240 ml of Part II.)

The preferred enzyme activity in the final multi-purpose solution will be about 5-25 PAU/ml.

What is Claimed is:

1. A two-compartment, sterile multi-purpose composition generating bottle assembly for use in cleaning, disinfecting, rinsing and storing a contact lens comprising:

a) a bottle containing an aqueous composition;

b) a sealed container comprising a break-away membrane and containing an enzyme composition containing an enzyme; and

c) an anti-microbial agent in an amount effective to disinfect the lens;

wherein the anti-microbial agent is included in either the aqueous composition or the enzyme composition, the container is secured to and partially within the bottle, forming an internally sterile, two-compartment bottle assembly and breaking away the membrane allows for sterile egress of the enzyme composition into the aqueous composition of the bottle, wherein a multi-purpose composition may be formed containing an amount of the enzyme effective to clean the lens and an amount of the anti-microbial agent effective to disinfect the lens.

2. A bottle assembly according to Claim 1, wherein:

the container further comprises a housing, a plunger, a removable cap and a collar;

the housing comprises a hollow cylinder containing the enzyme composition, a securing means, external threads and the membrane covers one end of the cylinder of the housing;

the plunger comprises a hollow cylinder, a dispensing end, and a bevel opposite end;

the cap comprises internal threads; and

the bottle further comprises an open end, neck and a receiving means for receiving and securing the container;

wherein the plunger is received within the hollow cylinder of the housing , the cap covers the plunger dispensing end and the internal threads of the cap are engaged with external threads of the housing, and the container is received and secured over the open end and within the neck of the bottle, forming an air-tight bottle assembly.

3. A bottle assembly according to Claim 1, wherein the enzyme is trypsin, subtilisin or a subtilisin BPN' variant.

4. A bottle assembly according to Claim 1, wherein the anti-microbial agent is polyquaternium-1.

5. A bottle assembly according to Claim 1, wherein the aqueous composition comprises polyquaternium-1, boric acid, sorbitol, sodium chloride, sodium citrate, Tetronic 1304, disodium edetate, AMP-95, MAPDA, sodium hydroxide, hydrochloric acid and water; and the enzyme composition comprises boric acid, glycerol, PEG-400, calcium chloride, water and an enzyme selected from the group consisting of trypsin, subtilisin and a subtilisin BPN' variant.

6. A bottle assembly according to Claim 5, wherein the aqueous composition comprises:

about 0.001% w/v of polyquaternium-1;

about 0.6% w/v of boric acid;

about 1.2% w/v of sorbitol;

about 0.65% w/v of sodium citrate;

about 0.1% w/v of sodium chloride;

about 0.05% w/v of Tetronic 1304;

about 0.05% w/v of disodium edetate;

about 0.45% w/v of AMP-95;

about 0.0005% w/v of MAPDA; and water; wherein the composition is adjusted to pH 7-8 with sodium hydroxide and hydrochloric acid.

7. A method of preparing a sterile multi-purpose composition which comprises:

employing a two-compartment bottle assembly, said bottle assembly comprising:

a) a bottle containing an aqueous composition;

b) a sealed container comprising a break-away membrane and containing an enzyme composition containing an enzyme; and

c) an anti-microbial agent;

wherein the anti-microbial agent is included in either the aqueous composition or the enzyme composition, and the container is secured to and partially within the bottle, forming an internally sterile, two-compartment bottle assembly;

breaking away the membrane and allowing the enzyme composition of the container to egress into the aqueous composition;

mixing the enzyme composition and aqueous composition together; and

forming a multi-purpose composition containing an enzyme in an amount effective to clean the lens and an anti-microbial agent in an amount effective to disinfect the lens.

8. A method according to Claim 7, wherein:

the container further comprises a housing, a plunger, a removable cap and a collar;

the housing comprises a hollow cylinder containing the enzyme composition, a securing means, external threads wherein the membrane covers one end of the cylinder of the housing;

the plunger comprises a hollow cylinder, a dispensing end, and a bevel opposite end;

the cap comprises internal threads; and

the bottle further comprises an open end, neck and a receiving means for receiving and securing the container;

wherein the plunger is received within the hollow cylinder of the housing, the cap covers the plunger dispensing end and the internal threads of the cap are engaged with external threads of the housing, and the container is received and secured over the open end and within the neck of the bottle, forming an air-tight bottle assembly.

9. A method according to Claim 7, wherein the enzyme is trypsin, subtilisin, or a subtilisin BPN' variant.

10. A method according to Claim 7, wherein the anti-microbial agent is polyquaternium-1.

11. A method according to Claim 7, wherein the aqueous composition comprises polyquaternium-1, boric acid, sorbitol, sodium chloride, sodium citrate, Tetronic 1304, disodium edetate, AMP-95, MAPDA, sodium hydroxide, hydrochloric acid and water; and the enzyme composition comprises boric acid, glycerol, PEG-400, calcium chloride, water and an enzyme selected from the group consisting of trypsin, subtilisin and a subtilisin BPN' variant.

12. A method according to Claim 11, wherein the aqueous composition comprises:

about 0.001% w/v of polyquaternium-1;

about 0.6% w/v of boric acid;

about 1.2% w/v of sorbitol;

about 0.65% w/v of sodium citrate;

about 0.1% w/v of sodium chloride;

about 0.05% w/v of Tetronic 1304;

about 0.05% w/v of disodium edetate;

about 0.45% w/v of AMP-95;

about 0.0005% w/v of MAPDA; and water; wherein the composition is adjusted to pH 7.8 with sodium hydroxide and hydrochloric acid.

13. A method of cleaning and disinfecting a contact lens which comprises:

preparing a sterile multi-purpose composition by employing a two-compartment bottle assembly comprising:

a) a bottle containing an aqueous composition;

b) a sealed container comprising a break-away membrane and containing an enzyme composition containing an enzyme; and

c) an anti-microbial agent;

wherein the anti-microbial agent is included in either the aqueous composition or the enzyme composition, and the container is secured to and partially within the bottle, forming an internally sterile, two-compartment bottle assembly;

breaking away the membrane and allowing the enzyme composition of the container to egress into the aqueous composition;

mixing the enzyme composition and aqueous composition together;

forming a multi-purpose composition containing an enzyme in an amount effective to clean the lens and an anti-microbial agent in an amount effective to disinfect the lens;

dispensing the multi-purpose composition into a receptacle; and

soaking the lens in the multi-purpose composition of the receptacle for a time sufficient to clean and disinfect the lens.

14. A method according to Claim 13, wherein:

the container further comprises a housing, a plunger, a removable cap and a collar;

the housing comprises a hollow cylinder containing the enzyme composition, a securing means, external threads wherein the membrane covers one end of the cylinder of the housing;

the plunger comprises a hollow cylinder, a dispensing end, and a bevel opposite end;

the cap comprises internal threads; and

the bottle further comprises an open end, neck and a receiving means for receiving and securing the container;

wherein the plunger is received within the hollow cylinder of the housing, the cap covers the plunger dispensing end and the internal threads of the cap are engaged with external threads of the housing, and the container is received and secured over the open end and within the neck of the bottle.

15. A method according to Claim 13, wherein the enzyme is trypsin, subtilisin or a subtilisin BPN' variant.

16. A method according to Claim 13, where the anti-microbial agent is polyquaternium-1.

17. A method according to Claim 13, wherein the aqueous composition comprises polyquaternium-1, boric acid, sorbitol, sodium chloride, sodium citrate, Tetronic 1304, disodium edetate, AMP-95, MAPDA, sodium hydroxide, hydrochloric acid and water; and the enzyme composition comprises boric acid, glycerol, PEG-400, calcium chloride, water and an enzyme selected from the group consisting of trypsin, subtilisin and a subtilisin BPN' variant.

18. A method according to Claim 17, wherein the aqueous composition comprises:

about 0.001% w/v of polyquaternium-1;

about 0.6% w/v of boric acid;

about 1.2% w/v of sorbitol;

about 0.65% w/v of sodium citrate;

about 0.1% w/v of sodium chloride;

about 0.05% w/v of Tetronic 1304;

about 0.05% w/v of disodium edetate;

about 0.45% w/v of AMP-95;

about 0.0005% w/v of MAPDA; and water; wherein the composition is adjusted to pH 7.8 with sodium hydroxide and hydrochloric acid.